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Short communication

Improving the efficacy of sewage treatment decreases norovirus contamination in oysters

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ABSTRACT

As human population increases worldwide, water quality will become increasingly problematic, and food consumed raw may be of higher risk. This is already evident for oysters grown in coastal areas - despite regulations based on bacterial indicators, oysters are still implicated in food-borne outbreaks worldwide. The pathogens most frequently detected are human noroviruses, which are shed at high concentrations in human excreta and are very resistant to environmental conditions. Sewage treatment plants usually apply a variety of steps such as activated sludge treatment, chlorine or UV disinfection to eliminate contaminants, these processes have variable efficacy. This study demonstrates the impact of replacing an old lagoon-based sewage treatment plant with a new membrane bioreactor sewage treatment plant on human norovirus levels in treated sewage and oysters. While comparable norovirus concentrations were detected in the influent samples, a clear difference was observed in effluent quality, as norovirus was only detected in one sample after treatment in the new membrane bioreactor system, confirming the efficiency of such technology. As a direct impact, oysters located close to the membrane bioreactor sewage outfall were less frequently contaminated by norovirus, and showed lower concentrations compared to the first period of the study when they were exposed to sewage effluent from the lagoon outfall. Shellfish located upstream showed comparable contamination levels suggesting that there are also other sources of norovirus contamination in the estuary. Considering the health benefits of shellfish consumption, improving wastewater quality will make an important contribution to enhancing the safety of shellfish and international food security.

1. Introduction

Contaminated food and water is a major pathway for the transmission of infectious diseases and poses a global health issue, with viruses being one of the most frequently reported infectious agents (Havelaar et al., 2015). Human effluent contains a large variety of microbial pathogens that are derived from the human gut, such as enteric viruses that cause gastroenteritis or hepatitis. These viruses are excreted in the stools and vomitus of infected individuals at high concentrations, thus wastewater effluent is a major source of viruses in environmental waterways (Monedero et al., 2018; Newton et al., 2015). Increasing viral elimination during sewage treatment should lead to improvements in effluent quality and help to prevent further distribution and transmission of viruses through the food chain, this is especially important for foods that are consumed raw (Uyttendaele et al., 2015). For example, the association between raw shellfish consumption and human viral diseases was recognized around 40 years ago (Murphy et al., 1979).

The viral pathogens most frequently responsible for shellfish-borne illness outbreaks are human noroviruses (HuNoV) (Yu et al., 2015). Within the *Caliciviridae* family, HuNoVs are non-enveloped viruses, with a single-stranded positive-sense RNA genome. They are classified into seven genogroups (G), three of which infect humans, GI, GII and GIV (De Graaf et al., 2016). Infection induces acute gastroenteritis with vomiting and diarrhea, with clinical symptoms lasting for 2 to 3 days and a large quantity of progeny viruses are shed in the feces of infected people for several weeks (Atmar et al., 2014; Teunis et al., 2015). Asymptomatic people can also shed high concentrations of viral particles in their stools, thus during gastroenteritis outbreaks large numbers of viral particles may be discharged into water bodies through the

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release of untreated or partially treated sewage (Sano et al., 2016; Teunis et al., 2015).

Sewage treatment plants usually apply a combined process to reduce contaminant levels, which may include physical (e.g.; settlement), biological (e.g. activated sludge) and chemical steps (e.g. chlorination) (Sano et al., 2016). The efficacy of each sewage treatment process (e.g. waste stabilization ponds, activated sludge or membrane bio-reactors) in removing viral contaminants varies, as does the impact on the coastal environment (Miura et al., 2018; Wang et al., 2018).

The objective of this study was to evaluate the impact of replacing an old sewage treatment plant with waste stabilization ponds (lagoon treatment) with a new sewage treatment plant with a membrane bioreactor on HuNoV levels in oysters that were strategically placed in the area for this study.

2. Materials and methods

2.1. Study site

The estuary is located in south western France (Brittany), and it contains a sewage treatment plant (STP) in close proximity to shellfish beds (Fig. 1). Before October 2013, the STP was composed of a gravity-fed lagoon treatment system which had a series of 3 ponds (also called a waste stabilization pond system). During 2013, a new STP with membrane bioreactor treatment (MBR) was built adjacent to the old STP and it was commissioned in October 2013.

For the study, oyster batches (around 500 individual oysters each) were placed on oyster tables (50 cm above the bottom of the estuary) at selected points around the STP outfall: three points (A, B, C) were located upstream (around 1.5 to 2 km) of the outfall, and two sampling points (D, E) were located downstream (from 0.2 to 1 km) (Fig. 1).

2.2. Sample collection

Influent and effluent samples were collected bi-monthly (monthly during the summer months) over a one year period from the old lagoon STP, and over a seven month period from the new STP. Automatic samplers collected 24-h composite samples with a total volume of 1-L. Oyster samples were collected the following day, from the five sampling points. The two periods for collecting the oyster samples (collection at low tide) were long enough and were over the same time period each the year (including winter and spring time), thus ensuring that other potential impacts from factors such as the tide, rain and wind were tacitly accounted for in the experimental design. All samples were transported to the laboratory on ice and processed within 24 h of collection. A total of 60 wastewater samples (30 influent and 30 effluent) and 148 oyster samples were collected.

2.3. Sample processing

Effluent samples were concentrated from 1 L to 40 mL with cross-flow ultrafiltration (Vivaflow 50, Sartorius, Germany) (Sima et al., 2011). Mengovirus (MgV) (2×10^6 RNA copies) was added to the effluent concentrate and to 40-mL of un-concentrated influent samples and then concentrated using a polyethylene glycol (PEG) precipitation method (Sima et al., 2011). The PEG pellet was suspended in 1 mL of deionized distilled water (DDW) with a vortex mixer.

For oyster samples, 10 individuals were shucked, weighed, and the digestive tissues (DTs) dissected. MgV (2×10^6 RNA copies) was added to each DT sample (2 g) before incubation with 2 mL of proteinase K solution (ISO/TS 15216-1, 2017).

2.4. Nucleic acid extractions

For wastewater samples, the re-suspended PEG pellet was mixed with 2 mL of lysis buffer (bioMérieux, Lyon France) (Sima et al., 2011). For oyster samples, after the proteinase K treatment the entire supernatant (around 3 mL) was mixed with 10 mL of lysis buffer (Le Mennec et al., 2017). Both mixtures were incubated for 10 min at room temperature, and after a brief centrifugation to eliminate solid particles (if

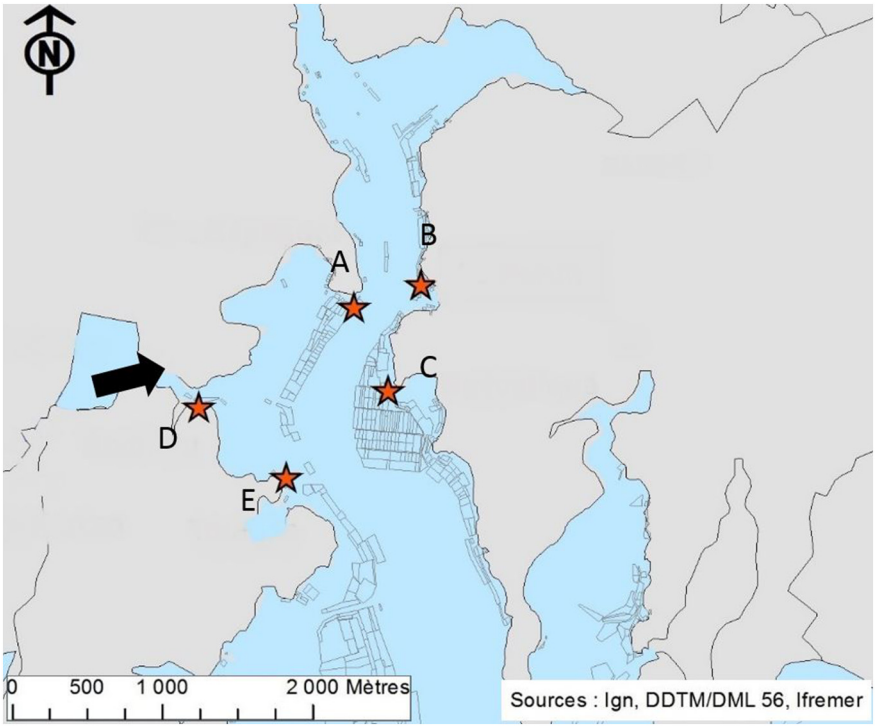

Fig. 1. Location of the sampling points in the estuary.
The red stars show the shellfish sample sites and the black arrow shows the sewage outfall location. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)